Examensarbeten **2013:11**
Institutionen för skogens ekologi och skötsel

Nitrogen fixation among boreal feather mosses along a clear-cut chronosequence

Kvävefixering hos de boreala mossorna ***Pleurozium schreberi*** *och* ***Hylocomium splendens*** *längs en kronosekvens skött med trakthyggesbruk*

Lisa Wik Persson

Sveriges Lantbruksuniversitet Jägmästarprogrammet
Examensarbete i biologi, 30 hp, avancerad nivå A2E ISSN 1654-1898
Handledare: Marie-Charlotte Nilsson Hegethorn , SLU, Inst för skogens ekologi och skötsel
Examinator: David Wardle, SLU, Inst för skogens ekologi och skötsel Umeå 2013

Examensarbeten **2013:11**
Institutionen för skogens ekologi och skötsel

Nitrogen fixation among boreal feather mosses along a clear-cut chronosequence

Kvävefixering hos de boreala mossorna Pleurozium schreberi och Hylocomium splendens längs en kronosekvens skött med trakthyggesbruk

Lisa Wik Persson

Nyckelord / *Keywords:*
Boreal forest, nitrogen fixation, feather moss, *Pleurozium schreberi, Hylocomium splendens,* cyanobacteria, acetylene reduction, Sweden / *Boreal skog, kvävefixering, Pleurozium schreberi, Hylocomium splendens, cyanobakterier, acetylen reduktion, Sverige*

ISSN 1654-1898 Umeå 2013

Sveriges Lantbruksuniversitet / *Swedish University of Agricultural Sciences*
Fakulteten för skogsvetenskap / *Faculty of Forest Sciences*
Jägmästarprogrammet / *Master of Science in Forestry*
Examensarbete i biologi / *Master degree thesis in Biology*
EX0705, 30 hp, avancerad nivå A2E/ *advanced level* A2E

Handledare / *Supervisor*: Marie-Charlotte Nilsson Hegethorn
SLU, Inst för skogens ekologi och skötsel / *SLU, Dept of Forest Ecology and Management*
Bitr handledare / *Assistant supervisor*: Babs Stuiver
SLU, Inst för skogens ekologi och skötsel / *SLU, Dept of Forest Ecology and Management*
Examinator / *Examiner:* David Wardle
SLU, Inst för skogens ekologi och skötsel / *SLU, Dept of Forest Ecology and Management*

I denna rapport redovisas ett examensarbete utfört vid Institutionen för skogens ekologi och skötsel, Skogsvetenskapliga fakulteten, SLU. Arbetet har handletts och granskats av handledaren, och godkänts av examinator. För rapportens slutliga innehåll är dock författaren ensam ansvarig.

This report presents an MSc/BSc thesis at the Department of Forest Ecology and Management, Faculty of Forest Sciences, SLU. The work has been supervised and reviewed by the supervisor, and been approved by the examiner. However, the author is the sole responsible for the content.

Abstract

The Swedish boreal forests are limited by nitrogen (N) availability. While biological N_2 fixation by cyanobacteria hosted by pleurocarpous feather mosses are important sources of N input to natural boreal forest ecosystems, little is known about the patterns of N_2 fixation in silvicultural systems. This study investigates the biological N_2 fixation rates of two boreal feather moss species (*Pleurozium schreberi* and *Hylocomium splendens*) along a chronosequence from clear-cut to mature forest, as well as the vegetation characteristics along the chronosequence. Measurements and samples were collected from 32 forest sites that were classified into four age classes (clear-cut, pre-commercially thinned, thinned and mature forest). The sites were located near the city of Arvidsjaur in northern Sweden. Moss, dwarf shrub and grass biomass were determined at each forest site and gametophytes of *P. schreberi* and *H. splendens* were collected for acetylene reduction analysis. The results showed that the biomass of *P. schreberi* was significantly higher in pre-commercially thinned and thinned stands than in clear-cuts while *H. splendens* had a much lower biomass that did not change over time. The total N_2 fixation rate varied between 0.1-1.4 kg N_2 ha^{-1} yr^{-1} and was highest in pre-commercially thinned and thinned stands. The results from this study shows that naturally occurring biological N_2 fixation vary manifold across managed forest stands. It also suggests that forest management can be used as a tool to modify biological N_2 input to N limited silvicultural systems, although further studies will be needed to determine its impact on soil fertility and effectiveness in supporting tree growth.

Keywords: Boreal forest, nitrogen fixation, feather moss, *Pleurozium schreberi*, *Hylocomium splendens*, cyanobacteria, acetylene reduction, Sweden

Sammanfattning

De svenska boreala skogarna är kvävebegränsade. Det är känt att biologisk N_2 fixering utförd av cyanobakterier med pleurokarpa mossor som värdar, är viktiga källor för kvävetillförsel i boreala skogsekosystem. Men det finns mycket lite kunskap om N_2 fixering i brukade skogar. Denna studie undersöker kvävefixeringen hos de två boreala mossarterna *Pleurozium schreberi* (väggmossa) och *Hylocomium splendens* (husmossa) längs med en kronosekvens från kalhygge till fullvuxen skog. Utöver kvävefixeringen beskrivs även vegetationskarakteristiska längs kronosekvensen. Vegetationsprover samlades in från 32 lokaler längs kronosekvensen som klassificerats i fyra åldersklasser (hygge, röjningsskog, gallringsskog och fullvuxen skog). Lokalerna var belägna nära Arvidsjaur i norra Sverige. Mängden biomassa hos mossa, bärris och gräs samlades in och bestämdes på varje lokal. Och gametofyter från *P. schreberi* och *H. splendens* samlades in för acetylen reduktionsanalys. Resultaten visade att *P. schreberi* var mer vanligt förekommande i röjnings- och gallringsskog än på hyggen medan *H. splendens* hade en mycket lägre biomassa som inte förändrades längs med kronosekvensen. Den totala kvävefixeringen varierade mellan 0,1-1,4 kg N_2 ha^{-1} yr^{-1} och var högst i röjnings- och gallringsskog. Resultaten från denna studie visar att naturligt förekommande N_2 fixering varierar mångfaldigt mellan brukade skogsbestånd. De indikerar också att skogsskötsel kan användas som ett verktyg för att modifiera biologisk kvävetillförsel till kvävebegränsade och brukade skogsekosystem. Dock behövs det fortsatta studier för att klargöra dess påverkan på markens bördighet och förmåga att gynna trädens tillväxt.

Nyckelord: Boreal skog, kvävefixering, *Pleurozium schreberi*, *Hylocomium splendens*, cyanobakterier, acetylen reduktion, Sverige

Table of contents

1 Introduction

1.1 Boreal forests

Northern Sweden is part of the northern coniferous forest or the boreal forest. Boreal forests are characterised by a low monthly average temperature, low annual precipitation (Townsend et al., 2008) and cover 33% of the world's landmass (Sands, 2007). The plant diversity is low compared to most other forest biomes due to that areas occupied by boreal forests were covered in ice during the last ice age (Townsend et al., 2008). In Sweden the two dominating coniferous tree species are Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) (Wigrup, 2012) while the ground vegetation is commonly dominated by ericaceous dwarf shrubs, lichen and bryophytes (SLU, 2011). Two very common bryophyte species are the feather mosses *Pleurozium schreberi* and *Hylocomium splendens* which in 2005 covered approximately 2500 and 1400 m^2/ha respectively, of productive Swedish forests (SLU, 2011). The northern boreal forest has two main types of natural disturbance; forest fire and wind (Sands, 2007), but the major disturbance is caused by humans in the form of silviculture.

1.2 The role of nitrogen in boreal forests

Most boreal forest ecosystems are strongly limited by nitrogen (N) and an increase of available N often results in increased productivity (Tamm, 1991) since N is an important component of proteins, amino- and nucleic acids (Campbell & Reece, 2005). It is assumed that this limitation is caused by slow mineralisation of soil organic N (Näsholm et al., 1998). The main biological input of N into the boreal ecosystem is through N_2 fixation where atmospheric N_2 is transformed by diazotrophs into forms that can be utilised by plants (Brady & Weil, 2008). A majority of the N_2 uptake in terrestrial ecosystems occur through symbiotic biological N_2 fixation (Brady & Weil, 2008). Symbiotic N_2 fixation has mainly been associated with primary or early secondary successional stages (Rastetter et al., 2001) and it has for long been thought that boreal forests lack wide-spread N_2 fixing plants (Tamm, 1991). However, it was recently discovered that both *P. schreberi* and *H. splendens* occupying late fire-successional sites host large populations of N_2 fixing cyanobacteria (DeLuca et al., 2002; Gundale et al., 2012; Lagerström et al., 2007; Zackrisson et al., 2009).

1.3 Cyanobacteria and nitrogen fixation of feather mosses

A range of different species of epiphytic cyanobacteria have been found on *P. schreberi* and *H. splendens* (DeLuca et al., 2002; Gentili et al., 2005; Ininbergs et al., 2011). The identified species of cyanobacteria include multiple strains of *Nostoc* sp., *Calothrix* sp. and *Stigonema* sp. as well as *Cylindrospermum* (DeLuca et al., 2002; Gentili et al., 2005; Houle et al., 2005; Zackrisson et al., 2009; Ininbergs et al., 2011). It is suggested that N_2 fixation rates of feather mosses are affected by various environmental factors such as moisture and N availability (Gundale et al., 2009, 2012; DeLuca et al., 2008; Jackson et al., 2010), and that changes in moisture availability has a larger impact on N_2 fixation in old forests compared to that in young forests (Gundale et al. 2009). The addition of anthropogenic N reduces fixation rates (Ackermann et al., 2012; Sorensen et al., 2012). N_2 fixation rates can also vary greatly with temperature and light intensity. Gentili et al. (2005) and Gundale et al. (2012) showed that optimal temperatures for fixation are cyanobacterial specific, implying that this could be an

effect of the physiological differences between different moss species and their associated cyanobacteria.

1.4 Forest management practises and its impact on ground layer vegetation

The Swedish forests are often managed for wood production. The conventional way of forest management is clear-cut followed by scarification and planting, when the forest is established most stands will be thinned at least a couple of times (Albrektson et al., 2012). In 2011, 942 000 ha out of Sweden's 22.5 million ha of productive forest land was either clear-cut, thinned or pre-commercially thinned (Wigrup, 2012). These management practises have a significant impact on the diversity and composition of understory plant communities (Bergstedt & Milberg, 2001). For example; *Vaccinium myrtillus,* the most dominant species in the field layer has been reported to decrease in abundance after clear-cutting while grasses increase or benefit from the open environment created by clear felling (Atlerim & Sjöberg, 1996; Bergstedt & Milberg, 2001). Clear-cut stands have also been found to have a lower moss cover compared to unmanaged forest (Uotila & Kouki, 2005).

1.5 Current knowledge

It was known for decades that N_2-fixing cyanobacteria are associated with mosses in subarctic wetland and polar ecosystems (Granhall & Selander, 1973; Christie, 1987; Chapin et al., 1991). However, in 2002 DeLuca et al. reported on a previously unknown association between the boreal feather moss *Pleurozium schreberi* and the epiphytic cyanobacteria *Nostoc* sp. Since then a number of studies have explored this association as well as made new findings of several other feather moss species associated with cyanobacteria. However, all these studies were conducted in forests uninfluenced by modern forest management (DeLuca et al., 2002, 2007; Gentili et al., 2005; Zackrisson et al., 2004, 2008; Gundale et al., 2009, 2010, 2012; Ackermann, 2012). In Sweden, about 20 million ha of productive forest land is managed (Wigrup, 2012) and little is known of how conventional forest management affect N_2 fixation of boreal feather mosses.

1.6 Goal & aim

The goal of this thesis was to examine the longer-term (0-42 years) effects of clear-cutting on the occurrence and N_2 fixation rates of feather mosses, and in this way determine whether N input to boreal forests through feather mosses changes with time following traditional forestry operations. For this work, a gradient of 24 production forest sites representing a 42 year old chronosequence generated by increasing time since clear-felling, and another eight sites consisting of 100-year old forests was used. The following two specific questions were asked:

1) How do the occurrence and N_2 fixation rates of *Pleurozium schreberi* and *Hylocomium splendens* change with time after clear-cutting?
2) What is the relative contribution of each moss species to overall N input since clear-felling?

In addition, the intention was to characterize the tree and understory vegetation along this chronosequence because these factors may have a role in controlling feather moss abundance and N_2 fixation rates.

The ultimate aim of this work is to provide fundamental knowledge that should enhance our understanding about how production forests impact on N_2 fixation rates of boreal feather mosses, and yield new insights as to how forest management practises can lead to N input of boreal forests.

2 Material & Methods

2.1 Study area

The study was conducted in the northern boreal forest zone, near the city of Arvidsjaur (65°35′N; 19°10′E) in northern Sweden (Figure 1). This area is part of the Baltic shield and dominated by acid intrusive rocks such as granite or acid volcanic rock such as rhyolite (SGU). Its' climate is classified as Dfc (cold, without dry season, cold summer) according to the Köppen-Geiger climate classification (Peel et al., 2007) with an annual (1961-2011) average temperature of 0.2°C and annual precipitation of 644mm (of which ~40% falls as snow) (SMHI, 2013-04-11). Logging on a commercial scale around Arvidsjaur begun in the middle of the 19th century but it was mostly dimension cutting where the largest trees would be removed first, followed by repeated logging of trees in decreasing sizes (Holmgren, 1959). Today, the dominating forest management is clear-cutting followed by soil scarification, regeneration, pre-commercial thinning and thinning (Albrektson et al., 2012).

Figure 1 *Map over Sweden, with the rectangle indicating the approximate study area.*

2.2 Site selection

Within this area 32 forest sites were selected, and of which 24 sites were clear-cuts and 8 sites consisted of mature forests. All sites were located on land owned by Sveaskog, situated between 380 and 530 meters above sea level on the same sandy silt glacial soil type and were classified as bilberry (*Vaccinium myrtillus*) vegetation type sites (Hägglund & Lundmark, 2004). The sites were classified into four different age classes, depending on the time since clear felling; sites that were recently clear-cut (0-6 years ago), sites that were cut 12-19 years ago (pre-commercial thinning stands), those that were cut 26-42 years ago (thinning stands) and those that have not been cut over the last 100 years (mature forest) (Figure 2). All sites represent typical stages of production forests found in Sweden. There were eight sites within each age group where each site represented an independent replicate. The tree layer in the mature forest (>100 yrs) consisted of a mixture of *Pinus sylvestris* and *Picea abies* while all of the other sites were planted with *P. sylvestris*.

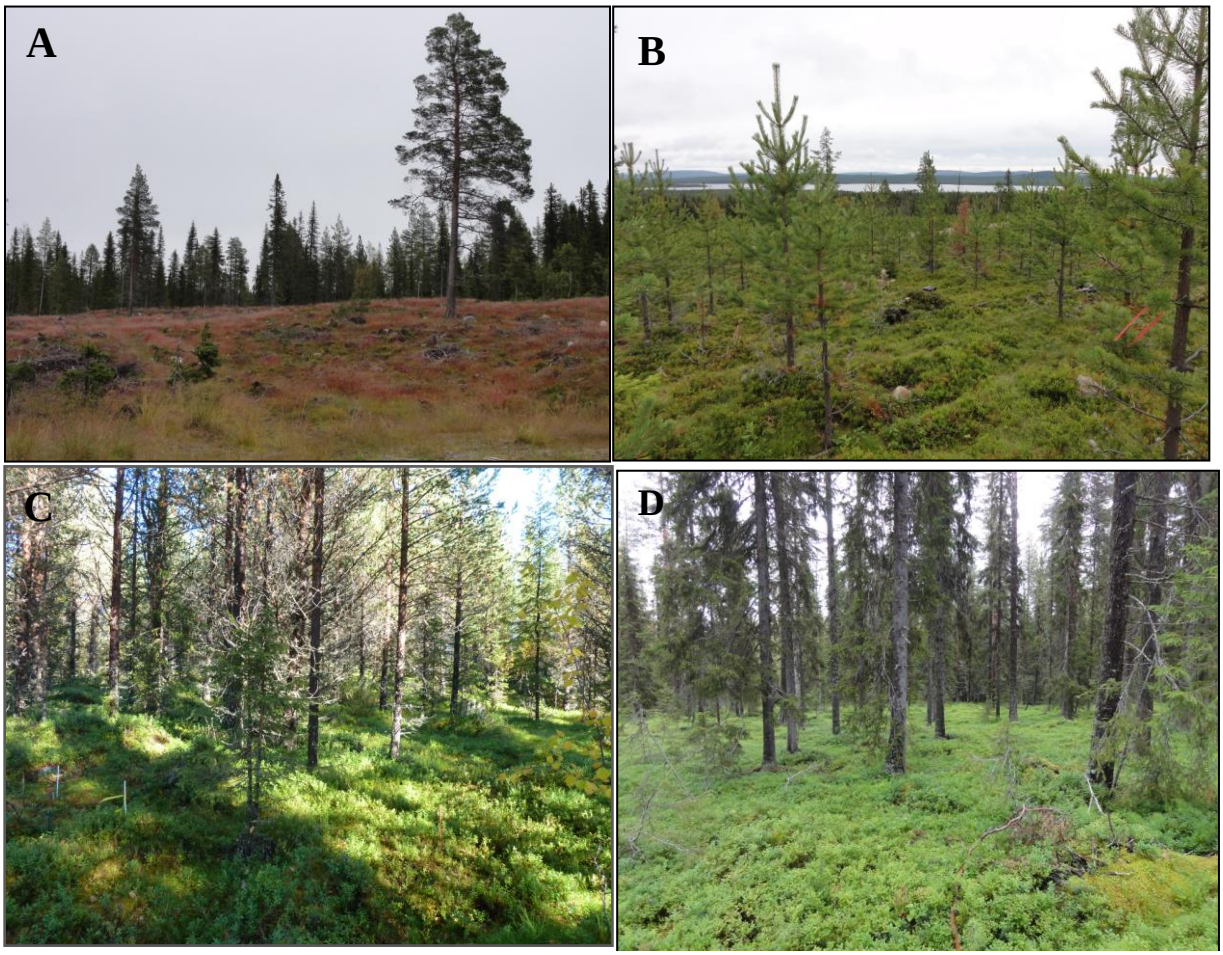

Figure 2 *Pictures of study sites showing: A) a clear-cut, B) a pre-commercial thinning, C) a thinning and D) a mature forest site.*

2.3 Recording of vegetation variables

All sampling took place August 27th – 31st 2012. For each forest site, average tree height and number of stems per hectare was quantified by recording the height of all trees and the number of tree stems in each of three random circular plots (r=5.64) covering an area of 100m^2 per site. At each site, another 20 plots (each 50x50cm) over an area of approximately 2500m^2 were randomly established. In each of these plots, the cover of each of the ericaceous dwarf shrubs (*Vaccinium myrtillus*, *Vaccinium vitis-idea*, *Empetrum hermaphroditum*), grasses (*Deschampsia flexuosa*) and feather moss species were recorded with the Braun-Blanquet visual estimation method (Poore, 1955) (Figure 3). From these values, mean cover for each species per site was calculated. For ten of these plots, a 25x25cm sub-plot was established in the plot center and the biomass of shrubs and grasses was recorded by collecting all alive, above ground plant tissue. The samples were sorted by species, dried for 48 hours at 70°C and weighed, and later used for calculating mean biomass per species and forest site. In addition, for each of these ten plots, the moss biomass was also recorded by gathering all mosses within a circular sample plot with a radius of 5.1 cm at the plot centre (Figure 3). The mosses were sorted by species, dried for 48 hours at 70°C and weighed. The weights were then used to calculate biomass per species per m^2.

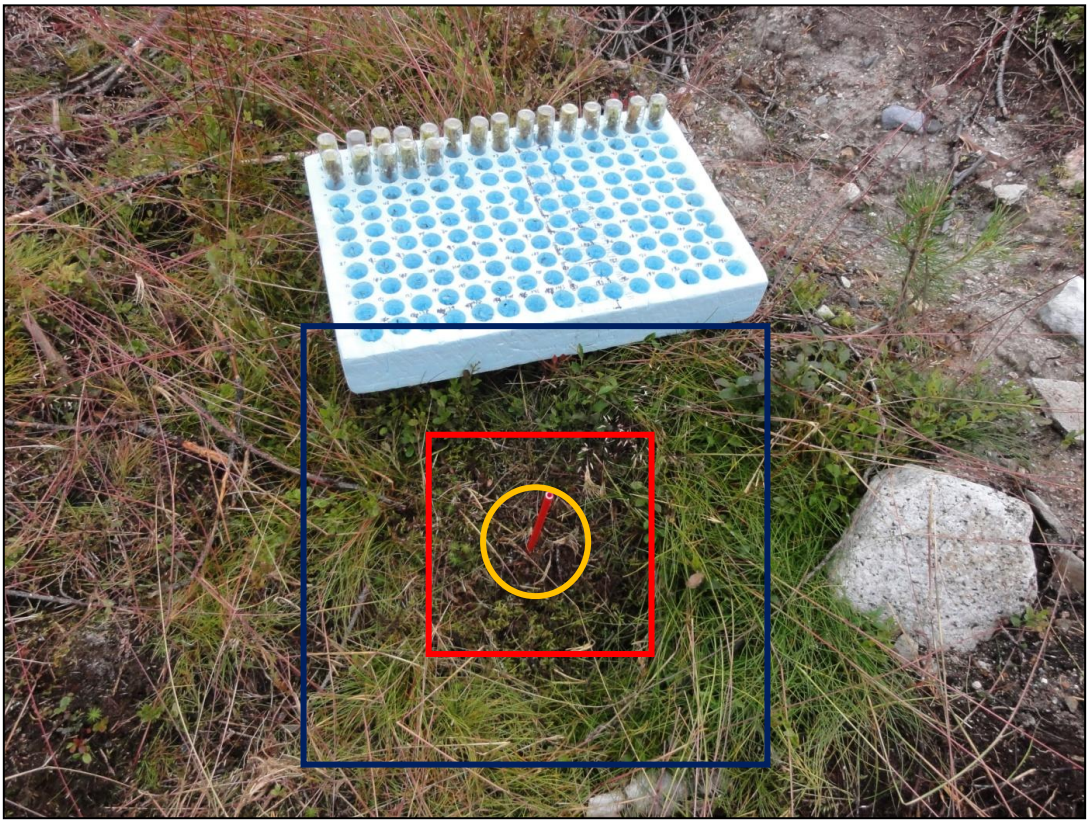

Figure 3 *Photograph of a plot used to calculate understory vegetation cover and biomass on a clear-cut site. The outer blue square depict the 50x50cm plot for measuring coverage (n per site=20), the inner red square the 25x25cm sub-plot for collecting shrub and grass biomass (n=10), and the yellow circle (r=5.1cm) the sub-plot for collecting moss biomass (n=10). The blue Styrofoam holds glass vials with feather mosses collected from the plot centre (n= 10) used for acetylene reduction analysis.*

2.4 N_2 fixation analysis

Sampling of feather mosses for acetylene reduction analysis was conducted in each of the ten sub-plots (see section 2.3) across the chronosequence to assess N_2 fixation rates. In each sub-plot, five gametophytes of *H. splendens* and ten of *P. schreberi* were randomly collected from as close to the sample plot centre as possible. Only the upper 2.5cm of the moss shoot was collected since most of the fixation occurs there (DeLuca et al., 2002). The gametophytes were placed in glass vials, with one vial for each plot and species (Figure 3). The samples were then transported to the laboratory and stored in moist conditions in a climate chamber at 20-13 °C (day:night), and 20 h of light and 4 h of darkness for 5-7 weeks until analysis. Prior to analysis each vial was sealed, 2ml of air was removed and 2ml of acetylene gas was injected into the vials 13h and 15min before they were run through the gas chromatograph. After the run, the samples were dried for 48h at 70°C and then weighed to determine the amount of mg N_2 fixed kg^{-1} moss d^{-1} for each moss species (Lagerström, 2007). In order to estimate N_2 fixation rates on a landscape basis, these values were upscaled to kg N_2 fixed ha^{-1}

yr^{-1} by using the moss biomass present at each stand and a growth period of 200 days following Lagerström (2007).

2.5 Statistics

In order to test for how chronosequence stages differed in the presence of different dwarf shrubs, grasses and feather mosses, and N_2 fixation rates a number of statistical tests were performed. First, to test the difference between the total biomass of mosses, dwarf shrubs and grasses a Kruskal-Wallis test together with Scheffe's post-hoc tests was used. Secondly, moss biomass data collected from each site was tested with two-way ANOVA and Tukey post-hoc, using age class and species as main factors. Third, to test for the variation within each species across the chronosequence, a one-way ANOVA and Tukey post-hoc test was performed to identify the significant differences. Biomass values of the mosses were square root +1 transformed, while shrub and grass biomass values were transformed by LOG10 to meet the requirements of normality. Finally, to test for the effect of chronosequence on rates of N_2 fixation per unit moss weight and per unit land area, non-parametric Kruskal-Wallis test with a Scheffe's post-hoc test was used for both *P. schreberi* and *H. splendens*, because the N_2 fixation data was not normal distributed and could not be transformed to meet the normal distribution requirements. ANOVA tests and the Tukey post-hoc test for ground vegetation biomass were done in Minitab 16. The Kruskal-Wallis tests and the Scheffe's post-hoc tests were done in IBM SPSS Statistics 19. For all analyses, individual forest sites served as the unit of replication, with a sample size of 8 for each of the four age classes.

3 Results

3.1 Vegetation characteristics along the chronosequence

A general description of the vegetation characteristics of each of the forest sites along the chronosequence is provided in Table 1. Typically, grasses (*D. flexuosa*) was mainly found on recently cut sites (0-6yrs), *V. vitis-idea* and *E. hermaphroditum* on pre-commercially thinned (12-19yrs) and thinned sites (26-42yrs) and *V. myrtillus* on thinned and mature forest sites (>100yrs). The moss cover of *P. schreberi* and *H. splendens* was lowest on clear-cut sites. The number of tree stems was highest on clear-cut sites with 1791 stems/ha and lowest on mature forest sites with 525 stems/ha. Meanwhile, the tree height was highest in the mature forest and lowest on newly planted clear-cuts.

Table 1 *Vegetation characteristics of the study sites. Data are means ± SE.*

Age class (yrs after felling)		**0-6**	**12-19**	**26-42**	**>100**
Management classification		Clear-cut	Pre-commercial thinning	Thinning	Mature
Mean elevation (m)		426	443	473	439
***P. sylvestris* density (stems/ha)**		1791±163	1645±155	1078±97	525±57
***P. sylvestris* tree height (cm)**		24±4	271±26	684±58	1725±37
Moss cover (%)	*Pleu*	28±6	40±3	39±4	36±5
	Hylo	6±2	9±2	12±3	22±5
Shrub and grass cover (%)	*Vm*	18±2	31±2	40±5	56±2
	Vvi	20±3	27±2	28±2	22±2
	Emp	12±31	27±5	22±4	10±3
	Desch	27±3	14±3	14±2	6±1

Pleu=Pleurozium schreberi, Hylo=Hylocomium splendens, Vm=Vaccinium myrtillus, Vvi=Vaccinium vitis-idea, Emp=Empetrum hermaphroditum, Desch=Deschampsia flexuosa

When the vegetation characteristics of the chronosequence was described by using plant biomass (g m^{-2}), the biomass of the three plant functional groups (mosses, dwarf shrubs and grasses) all differed significantly from each other ($F=59.308$, $p=0.000$) with dwarf shrubs having the overall highest biomass across the chronosequence (Figure 4). Total shrub biomass was significantly lower in the clear-cut sites compared to the older stages of the chronosequence ($F=7.72$, $p=0.001$). In contrast to the mosses and shrubs, which were more abundant on sites older than 12 years, the grass *D. flexuosa* had its highest biomass on the younger clear-cuts and it decreased significantly with increasing time since clear-felling ($F=18.29$, $p=0.000$) (Figure 4). Two-way ANOVA test performed on age class and moss species showed an interaction (Table 2) because the total moss biomass on the clear-cuts were

lower than in the pre-commercial thinning and thinning sites, but the difference was non-significant compared to the mature forest sites (F=4.99, p=0.007) (Figure 4).

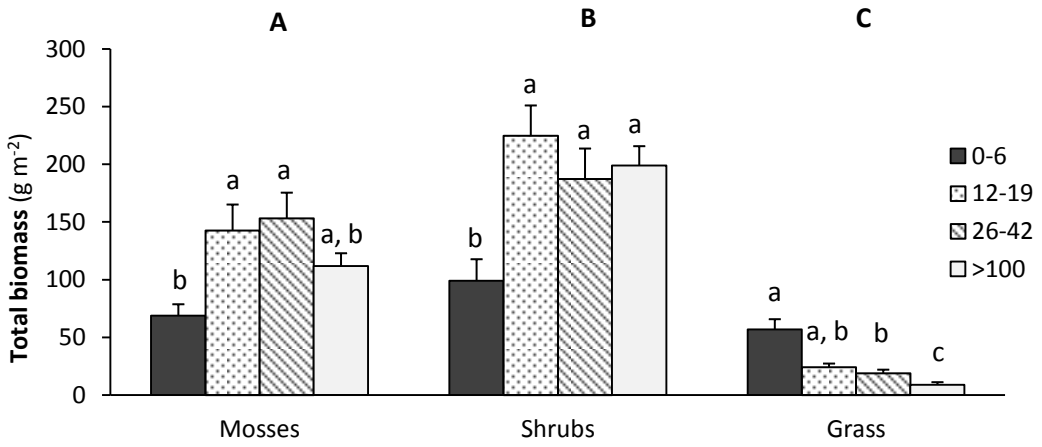

Figure 4 *The effect of stand age on total mean of feather moss, shrub and grass biomass (g m^{-2}). Mosses include* P. schreberi *and* H. splendens, *shrubs include* Vaccinium myrtillus, Vaccinium vitis-idea *and* Empetrum hermaphroditum, and g*rasses include* Deschampsia flexuosa. *Upper case letters express significant differences between functional groups and lower case letters express significant differences between age classes within each plant functional group. Scheffe's post-hoc test, p values are significant at α=0.05. Error bars show +1 SE.*

P. schreberi was significantly more abundant than *H. splendens* throughout the chronosequence (Figure 5, Table 3), and was significantly lower in the clear-cut stand compared to the other stands except for the mature forest. The amount of *P. schreberi* biomass in thinned sites was 155% higher compared to clear-cut sites. For *H. splendens* there was no change in biomass over the age classes (Table 3, Figure 5).

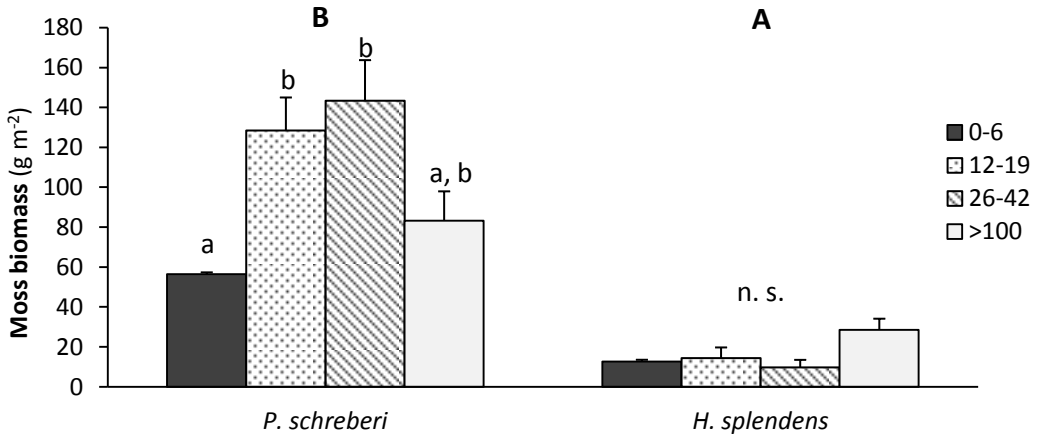

Figure 5 *The effect of stand age on total mean of moss biomass (g m^{-2}) of* P. schreberi *and* H. splendens. *Upper case letters express significant difference between feather moss species and lower case letters express significant differences between age classes within species (Table 3) following Tukey's post-hoc test. p values are significant at α=0.05. Error bars show +1 SE.*

The biomass of dwarf shrubs differed significantly from each other (Figure 6) with *V. myrtillus* having the overall highest biomass and *E. hermaphroditum* the lowest. There was no significant difference in biomass between the chronosequence stands for *V. vitis-idea* (F=2.26, p=0.104) or *E. hermaphroditum* (F=3.03, p=0.046) while *V. myrtillus* was significantly lower on clear-cuts compared to all other sites (F=6.17, p=0.002) (Figure 6).

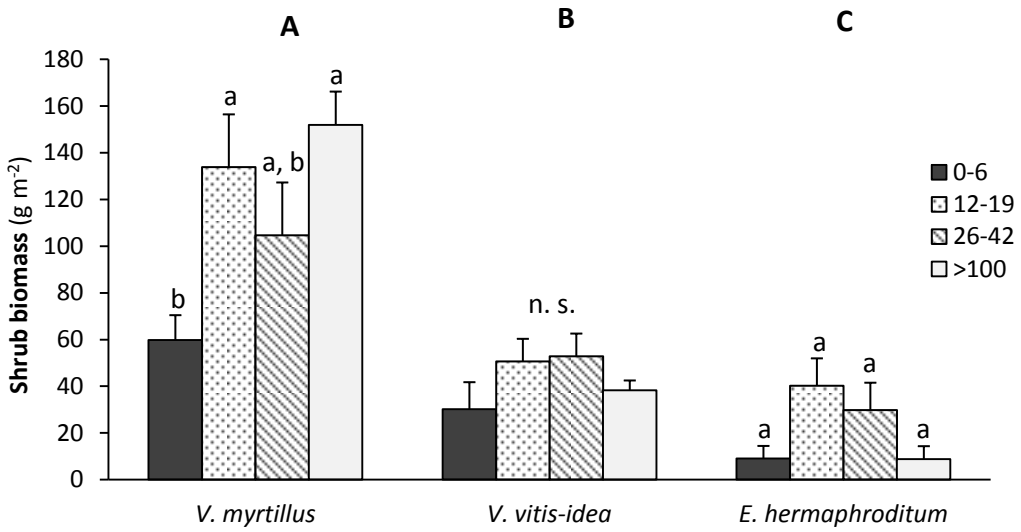

Figure 6 *The effect of stand age on total mean shrub biomass (g m^{-2}). Lower case letters express significant differences between age classes within species and upper case letters express significant difference between species (F=40.18; p=0.0000) following Tukey's post-hoc test, p values are significant at α=0.05. Error bars show +1 SE.*

3.2 N_2 fixation

There was no overall difference in N_2 fixation rates per unit moss weight and day between the feather moss species although there was a significant effect of stand age of N_2 fixation rates of both *P. schreberi* and *H. splendens* (Figure 7, Table 3). The mean N_2 fixation rate was highest for both moss species in the pre-commercially thinned sites, where *P. schreberi* and *H. splendens* fixed 4.6 and 6.4 mg N_2 kg^{-1} moss d^{-1} respectively. The fixation rates for both moss species were significantly lower at the other sites (Figure 7).

Table 2 *Results of two-way ANOVA test for moss biomass (g m^{-2}) and Kruskal-Wallis tests run across moss species and across all age classes (time after felling) for N_2 fixation per unit moss weight (mg kg^{-1} moss d^{-1}) and per unit land area (g ha^{-1} d^{-1}). n=8*

	Moss biomass (g m^{-2})*		**N_2 fixed (mg kg^{-1} moss d^{-1})**		**N_2 fixed (kg ha^{-1} yr^{-1})**	
	F	p	F	p	F	p
Age class	4.8	**0.005**	23.957	**0.000**	12.010	**0.007**
Species	100.66	**0.000**	3.552	0.059	5.010	**0.025**
Age class x species	6.52	**0.001**	-	-	-	-

*Two-way ANOVA Time after felling dF=3, Species dF=1

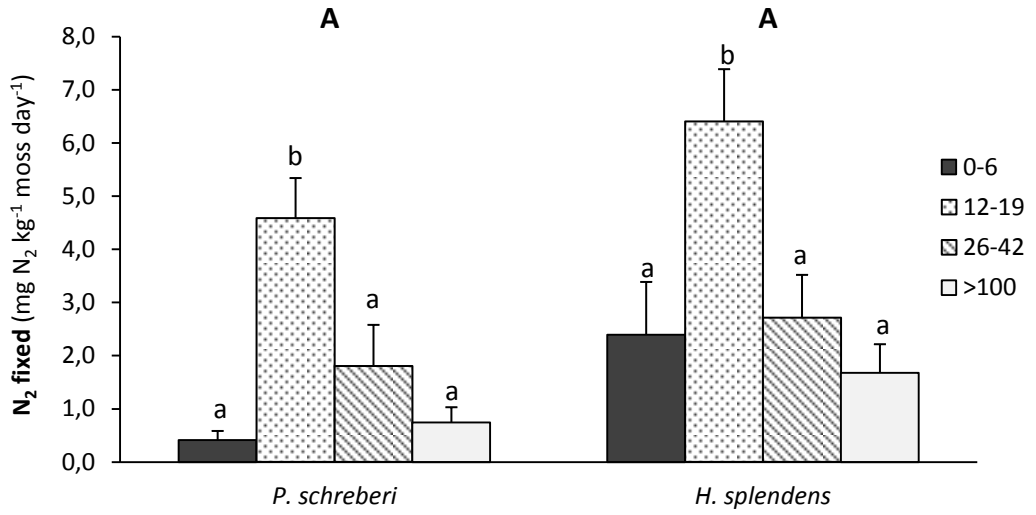

Figure 7 *Mean N_2 fixation capacities on a moss mass basis of* P. schreberi *and* H. splendens *(mg N_2 kg^{-1} moss d^{-1}) in response to stand age class. Lower case letters express significant differences between age classes within species (Table 3) and upper case letters express significant difference between species and. Scheffe's post-hoc test, p values are significant at α=0.05. Error bars show +1 SE.*

Table 3 *Results of Kruskal-Wallis tests run for the variable age class for each moss species specifically, N_2 fixation per unit moss weight (mg kg^{-1} moss d^{-1}) and per unit land area (g ha^{-1} d^{-1}), and biomass (g m^{-2}). One-way ANOVA test was used for biomass. Total=the added values of* P. schreberi *and* H. splendens. *n=8.*

	Moss biomass (g m^{-2})*		**N_2 fixed (mg kg^{-1} moss d^{-1})**		**N_2 fixed (kg ha^{-1} yr^{-1})**	
	F	p	F	p	F	p
Pleurozium	6.08	**0.003**	16.689	**0.001**	16.621	**0.001**
Hylocomium	2.78	0.059	10.188	**0.017**	1.229	0.746
Total	4.99	**0.007**	13.684	**0.003**	15.126	**0.002**

*One-way ANOVA Time after felling dF=3

When the fixation rates of the two feather moss species was expressed on a stand aerial bases (e.g. as mean kg N_2 per hectare and year), the highest total fixation was found in pre-commercially tinned sites, although this was only significantly different from that in clear-cuts and mature forests (Figure 8, Table 3). The same pattern emerged for *P. schreberi* which fixed significantly more N_2 per ha and year in the pre-commercially thinned sites compared to clear-cuts and mature forest sites (Figure 8) and the rates were 27 times higher in pre-commercially thinned sites compared to clear-cuts. The fixation rate per ha and year of *H. splendens* did not change with time since clear-felling

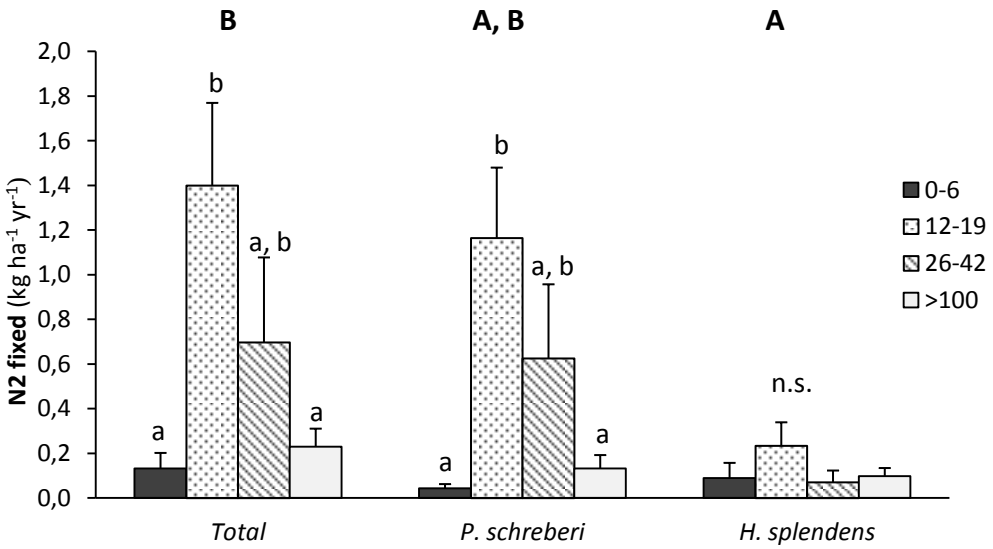

Figure 8 *Effect of forest stand age on mean N_2 fixation rates on a land area basis (kg N_2 ha^{-1} yr^{-1}) of* P. schreberi *and* H. splendens *and on N_2 fixation rates of both* P. schreberi *and* H. splendens (total). *Lower case letters express significant differences between age classes within group of mosses (Table 3) and upper case letters express significant difference between groups and Scheffe's post-hoc test, p values are significant at α=0.05. Error bars show +1 SE.*

4 Discussion

This study is the first to provide data on biological N_2 fixation of the two feather mosses *P. schreberi* and *H. splendens* in boreal forest sites managed for wood production, and show that the fixation rates are substantial in these forests. Previous studies on N_2 fixation by feather mosses in boreal forests have only taken place in unmanaged forest stands and chronosequences after natural disturbances (forest fire) (DeLuca et al., 2007; Gentili et al., 2005; Gundale et al., 2009, 2010; Lagerström et al., 2007; Markham, 2007; Zackrisson et al., 2004, 2009). Since a vast majority of the Swedish forests are managed for wood production (Wigrup, 2012), it is likewise important to study the potential for N_2 fixation by feather mosses in forests exposed to clear cutting and other methods typical for commercial forestry. The results emerging from this study show total N_2 fixation rates between 0.1 – 1.4 kg N_2 ha^{-1} yr^{-1} , values that are in the same range to what previously have been found in studies on unmanaged forests (DeLuca et al., 2002; Lagerström et al., 2007; Zackrisson et al., 2007, 2009).

Clear-cutting results in a rapid and dramatic change of the environment which might affect the occurrence and N_2 fixation rates of *P. schreberi* and *H. splendens*. In this work, the occurrence (biomass) of *P. schreberi* was lowest in the clear-cuts but increased with time since felling, and was significantly higher in the pre-commercially thinned and thinned stands. *H. splendens* had a much lower occurrence than *P. schreberi* and the biomass did not change over the chronosequence. The reason for the difference between the two species is not studied, but *P. schreberi* is known to occupy a wider range of microhabitats, is more tolerant to stress and recovers faster from changing environmental conditions, compared to *H. splendens* (Mäkipää & Heikkinen, 2003). The N_2 fixation rates on a mass basis (mg N_2 kg^{-1} moss d^{-1}) did not differ significantly between the moss species, indicating that the two mosses are equally efficient in fixing N_2. This finding contrasts with the results of Gundale et al. (2012) where *P. schreberi* fixed more than *H. splendens* at Time 0, and might be due to the fact that they kept the moss samples in a climate chamber for only one week compared to the present study where the moss samples were kept in the climate chamber for five to seven weeks prior to analysis. The longer time spent at stable environmental conditions might have given *H. splendens* a better chance to recover from the stress of changing environmental conditions during sampling. There was neither any significant difference in N_2 fixation rates on an aerial basis (kg N_2 ha^{-1} yr^{-1}) between *P. schreberi* and *H. splendens*. This occurs despite the dominance of *P. schreberi* biomass over that of *H. splendens* biomass, because the two species had the same N_2 fixation capacities on a mass basis and the difference in biomass was not enough to negate this. Both feather moss species had highest fixation rates in pre-commercially thinned sites, due to the fact that these sites have the environmental conditions most beneficial for both the mosses and their associated cyanobacteria.

When a stand is clear-cut it goes from a mature forest to an open area in a matter of days. This provides a rapid and radical change in environmental conditions for the ground vegetation, which are especially severe for mosses since they are poikilohydric and rely on atmospheric precipitation for water uptake (Désamoré et al., 2012). Clear-cuts experience more extreme

surface temperatures, more shortwave irradiance, increased wind speeds, lower humidity and higher soil temperature (Geiger, 1965 see Carlson & Groot, 1997; Lee, 1978 see Carlson & Groot, 1997; Stoutejesdijk & Barkman, 1992 see Carlson & Groot, 1997) as well as short term increase in N (Jerabkova et al., 2011). It has previously been suggested that light, temperature and moisture availability can affect N_2 fixation rates (DeLuca et al., 2008; Gentili et al., 2005; Gundale et al., 2009, 2012; Jackson et al., 2010) and it is likely that the change of these factors have a negative effect on N_2 fixation rates on clear-cuts. As the clear-cuts were forested again, the N_2 fixation rates increased greatly in only a few years from clear-cuts to pre-commercially thinned stands. However, the fixation rates was lower for the thinned and mature forest stands indicating that light limitation that increases with canopy closure (Table 1) might have contributed to these effects. It is also known from Canadian forests that high litter fall that deposits on the moss surface can have detrimental effects on the moss performance (Startsev et al., 2008). An increase in N input reduces moss density and abundance (Forsum, 2008). Further, nutrient availability (especially N) via canopy through fall or litter fall might also have down regulated fixation rates in these stands, as it is known that N_2 fixation is sensitive to N availability (DeLuca et al., 2008). Interestingly, these findings contradict those of previous studies that showed that fixation rates of feather mosses increases with age in natural successions after fire (Gundale et al., 2009; Lagerström et al., 2007; Zackrisson et al., 2004). There might be several reasons for this discrepancy. For example, the current study was performed on more fertile sites and previous management history is unknown. Also, the youngest stands studied during the post-fire successions were in the same age class, or older, as the thinned stands in this study (Gundale et al., 2009; Zackrisson et al., 2004). The youngest stands in Lagerström at al. (2007) had a mean age of 585 years and this difference in chronosequence age could affect the results. However, these findings require further studies in order to precisely determine the reasons for why old production forests appears to differ from that of late fire successional sites.

P. schreberi had a N_2 fixation rate on an aerial basis that was 27 times higher in in pre-commercially thinned sites compared to clear-cuts while *H. splendens* showed no significant change. This difference between how the N_2 fixation of the mosses varies over the chronosequence is probably determined by their biomass. The results of the present study indicate that N_2 fixation *P. schreberi* probably can adapt to changes in microclimate during chronosequence in a more efficient way then *H. splendens* and are in line with the results of Gundale et al. (2012). It is possible that the difficulties of *H. splendens* to recover its fixation rates on an aerial basis is due the species low resistance and resilience towards stress (Mäkipää & Heikkinen, 2003) and that this makes the moss less ‘attractive’ for cyanobacteria, which in turn gives *H. splendens* less N for growth.

Since the moss and dwarf shrubs show the same general pattern as the total N_2 fixation rate with a rapid increase in biomass from clear-cut to pre-commercially thinned stands it seems likely that this also is due to microclimate improvement. It could be that the presence of the dwarf shrubs helps to ameliorate moss growth and N_2 fixation by providing shelter and retaining moisture, while the trees are still small enough that they have little or no negative

effect. The presence of established trees with living roots could also be a factor since Gundale et al. (2010) found that lack of tree roots reduced N_2 fixation.

5 Conclusions

This investigation is the first where N_2 fixation by feather mosses in boreal forests managed for wood production has been studied. The results show that N_2 fixation occurs in commercially managed forests in magnitudes lower, but comparable to what have been previously recorded in natural forests. However, N_2 fixation rates are low during the clear-cut stages but N input via biological N_2 fixation in pre-commercially thinned stands are comparable to those reported for early fire successions. These findings suggest that forest management can be used as a tool to modify biological N_2 input to N limited silvicultural systems. Combined with further studies on how it impacts soil fertility and its effectiveness in supporting tree growth, managing for increasing biological N_2 fixation could be an interesting aspect in times when increased forest productivity for bioenergy is in high demand.

Acknowledgement

Thanks to my supervisor Marie-Charlotte Nilsson Hegethorn and associated supervisor Babs Stuiver for all their help, support and patience during this entire process. A great thanks to Kelley Gundale for her help with the acetylene reduction analysis.

References

Ackermann, K., Zackrisson, O., Rousk, J., Jones, D. L. & DeLuca, T. (2012). N_2 fixation in feather mosses is a sensitive indicator of N deposition in boreal forests. *Ecosystems*, **15**, 986-998.

Albrektson, A., Elfving, B., Lundqvist, L. & Valinger, E. (2012). *Skogsskötselserien – Skogsskötselns grunder och samband,* 2nd ed. Available: http://www.skogsstyrelsen.se/skogsskotselserien [2013-04-03]

Atlerim, O. & Sjöberg, K. (1996). Response of bilberry (*Vaccinium myrtillus*) to clear-cutting and single-tree selection harvests in uneven-aged boreal *Picea abies* forests. *Forest Ecology and Management*, **86**, 39-50.

Bergstedt, J. & Milberg, P. (2001). The impact on logging intensity on field-layer vegetation in Swedish boreal forests. *Forest Ecology and Management*, **154**, 105-115.

Brady, N. C. & Weil, R. R. (2008). *The nature and properties of soils,* 14th ed. USA: Pearson Education Inc.

Campbell, N. & Reece, J. (2005). *Biology.* 7th international ed. San Francisco: Pearson Education Inc.

Carlson, D. W. & Groot, A. (1997). Microclimate of clear-cut, forest interior, and small openings in trembling aspen forest. *Agricultural and Forest Meteorology*, **87**, 313-329.

Chapin, D. M., Bliss, L. & Bledsoe, L. (1991). Environmental regulation of nitrogen fixation in a high arctic lowland ecosystem. *Canadian Journal of Botany*, **69**, 2744-2755.

Christie, P. (1987). Nitrogen in two contrasting Antarctic bryophyte communities. *Journal of Ecology*, **75**, 73-79.

Désamoré, A., Laenen, B., Stech, M., Papp, B., Hedenäs, L., Mateo, R. G. & Vanderpoorten, A. (2012). How do temperate bryophytes face the challenge of a changing environment? Lessons from the past and predictions for the future. *Global Change Biology*, **18**, 2915-2924.

Forsum, Å. (2008). *Nitrogen enrichment of a boreal forest.* Diss. Swedish University of Agricultural Sciences. Umeå

Gentili, F., Nilsson, M.-C., Zackrisson, O., DeLuca, T. H. & Sellstedt, A. (2005). Physiological and molecular diversity of feather moss associative N_2-fixing cyanobacteria. *Journal of Experimental Botany*, **422**, 3121-3127.

Granhall, U. & Selander, H. (1973). Nitrogen fixation in a subarctic mire. *Oikos*, **24**, 8-15.

Gundale, M. J., Gustafsson, H. & Nilsson, M.-C. (2009). The sensitivity of nitrogen fixation by a feathermoss-cyanobacteria association to litter and moisture variability in young and old boreal forests. *Canadian Journal of Forestry Research*, **39**,

Gundale, M. J., Nilsson, M., Bansal, S. & Jäderlund, A. (2012). The interactive effects of temperature and light on biological nitrogen fixation in boreal forests. *New Phytologist*, **194**, 453-463.

Hallsby, G. (2013). *Skogsskötselserien – plantering av barrträd.* 2nd ed. Available: http://www.skogsstyrelsen.se/skogsskotselserien [2013-05-06]

Holmgren, A. (1959). Skogarna och deras vård i övre Norrland intill år 1930. In: Arpi, G. (ed), *Sveriges skogar under 100 år, del II*. p. 375-412. Stockholm: Kungliga Domänstyrelsen.

Houle, D., Bilodeau Gauthier, S., Paquet, S., Planas, D. & Warren, A. (2005). Identification of two genera of N_2-fixing cyanobacteria growing on three feather moss species in boreal forests of Quebec, Canada. *Canadian Journal of Botany*, **84**, 1025-1029.

Hägglund, B. & Lundmark, J.-E. (2004). *Bonitering del 3: Markvegetationstyper – skogsmarksflora.* 4th ed. Jönköping: Skogsstyrelsen.

Ininbergs, K., Bay, G., Rasmussen, U., Wardle, D. A. & Nilsson, M.-C. (2011). Composition and diversity of nifH genes of nitrogen-fixing cyanobacteria associated with boreal forest feather mosses. *New Phytologist*, **192**, 507-517.

Jackson, S. A., Fagerlund, R. D., Wilbanks, S. M. & Eaton-Rye, J. J. (2010). Crystal structure of PsbQ from *Synechocystis* sp. PCC 6803 at 1.8 Å: Implications for binding and function in cyanobacterial photosystem II. *Biochemistry*, **49**, 2765-2767.

Jackson, B. G., Martin, P., Nilsson, M.-C. & Wardle, D. A. (2011). Response of feather moss associated N_2 fixation and litter decomposition to variations in simulated rainfall intensity and frequency. *Oikos*, **120**, 570-581.

Jerabkova, L., Prescott, C. E., Titus, B. D., Hope, G. D. & Walters, M. B. (2011). A meta-analysis of the effects of clear-cut and variable-retention harvesting on soil nitrogen fluxes in boreal and temperate forests. *Canadian Journal of Forestry Research*, **41**, 1852-1870.

Lagerström, A., Nilsson, M.-C., Zackrisson, O. & Wardle, D. A. (2007). Ecosystem input of nitrogen through biological fixation in feathermosses during ecosystem regression. *Functional Ecology*, **21**, 1027-1033.

Markham, J. (2009). Variation in moss-associated nitrogen fixation in boreal forest stands. *Oecologica*, **161**, 353-359.

Mäkipää, R. & Heikkinen, J. (2003). Large-scale changes in abundance of terricolous bryophytes and macrolichens in Finland. *Journal of Vegetation Science*, **14**, 497-508.

Näsholm, T., Ekblad, A., Nordin, A., Giesler, R., Högberg, M & Högberg, P. (1998). Boreal forest plants take up organic nitrogen. *Nature*, **392**, 914-916.

Peel, M. C., Finlayson, B. L. & McMahon, T. A. (2007). Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences*, **11**, 1633-1644.

Poore, M. E. D. (1955). The use of phytosociological methods in ecological investigations: I. The Braun-Blanquet system. *Journal of Ecology*, **43** (1), 226-244.

Rastetter, E. B., Vitousek, P. M., Field, C., Shaver, G. R., Herbert, D. & Ågren, G. I. (2001). Resource optimization and symbiotic nitrogen fixation. *Ecosystems*, **4**, 369-388.

Sands, R. (2007). *Forestry in a global context.* United Kingdom: CAB International.

SGU – Sveriges Geologiska Undersökning. *Kartgeneratorn*. http://www.sgu.se/sgu/sv/produkter-tjanster/sgu_publ/index.html [2013-04-11]

SLU – Sveriges Lantbruksuniversitet (2011). *Skogsdata 2011: Aktuella uppgifter om de svenska skogarna från Riksskogstaxeringen.* Umeå.

SMHI – Sveriges Meterologiska och Hydrologiska Institut. *Klimatdata*. http://www.smhi.se/klimatdata [2013-04-11]

Sorensen, P. L., Lett, S. & Michelsen, A. (2012). Moss-specific changes in nitrogen fixation following two decades of warming, shading and fertilizer addition. *Plant Ecology*, **213**, 695-706.

Startsev, N., Victor, L. J. & Landhäusser, S. M. (2008). Effects of leaf litter on the growth of boreal feather mosses: Implication for forest floor development. *Journal of Vegetation science*, **19**, 253-260.

Steward, K. J., Lamb, E. G., Coxson, D. S. & Siciliano, S. D. (2011). Bryophyte-cyanobacterial associations as a key factor in N_2-fixation across the Canadian Artic. *Plant Soil*, **344**, 335-346.

Townsend, C. R., Begon, M. & Harper, J. H. (2008). *Essentials of ecology*. 3rd ed. Oxford: Blackwell Publishing.

Uotila, A. & Kouki, J. (2005). Understory vegetation in spruce-dominated forests in eastern Finland and Russian Karelia: Successional patterns after anthropogenic and natural disturbances. *Forest Ecology and Management*, **215**, 113-137.

Wigrup, I. (2012) (ed.) *Skogsstatistisk årsbok.* Skogsstyrelsen, Jönköping.

Zackrisson, O., DeLuca, T. H., Nilsson, M.-C., Sellstedt, A. & Berglund, L. M. (2004). Nitrogen fixation increases with successional age in boreal forests. *Ecology*, **85(12)**, 3327-3334.

Zackrisson, O., DeLuca, T. H., Gentili, F., Sellstedt, A. & Jäderlund, A. (2009). Nitrogen fixation in mixed *Hylocomium splendens* moss communities. *Oecologica*, **160**, 309-319.

SENASTE UTGIVNA NUMMER

2012:19 Författare: Caroline Pöntynen Boström
Röjningsplan för Sveaskog

2012:20 Författare: Robyn Hooper
Climate change impacts and forest management adaptation measures in Sweden and British Columbia, Canada: A case study of Swedish forest managers

2012:21 Författare: Addisu Almaw Semeneh
Effects of trees and termite nests in agroforestry parklands on preferential water flows: image analysis of soil profiles after rain simulations and dye experiments

2012:22 Författare: Torun Bergman
Skogsutnyttjandet vid den medeltida masugnen i Hyttehamn

2012:23 Författare: Johan Bäckman
Umebors åsikter rörande grönområden

2012:24 Författare: Andreas Engström
Insekter i hårt törskateangripna ungtallbestånd i Norrbotten. Skadeinventering och artbestämning

2013:1 Författare: Jenny Nilsson
Biogallring – effektivitet och lönsamhet vid gallring i ung skog

2013:2 Författare: Vidar Sjögren
Naturlig föryngring efter markberedning med harv eller Bracke Planter i Småland

2013:3 Författare: Hanna Jönsson
Kan vistelse i skogs- eller hantverksmiljö sänka stressade personers stressnivå? En jämförande studie

2013:4 Författare: Sven-Erik Zimmer
Effekter av höggallring i flerskiktad skog - beståndsutveckling i ett fältförsök med Naturkultur

2013:5 Författare: Javier Segura Angulo
Autumn water sources for understory vegetation and fungi in a boreal forest: An evaluation using stable isotypes

2013:6 Författare: Frida Snell
Bevarande av hotade epifytiska lavar och vedsvampar i Norrbottens län. – Rödlistan som verktyg i praktisk naturvård

2013:7 Författare: Ebba Okfors
Ekoturism i jordbrukslandskap – ett vinnande koncept? En tvärvetenskaplig studie om kulturvärden och naturvärden på Sjögetorp

2013:8 Författare: Anna Hallmén
Hur kan mångfalden gynnas på SCA:s naturvårdsareal? Natur- och kulturvärden i Peltovaara mångfaldspark

2013:9 Författare: Mattias Söderholm
Verktyg och metoder för kontroll av dubbskadedjup på timmerstockar - metodutveckling

2013:10 Författare: Johan Karlsson
Modellering av diametern hos tall (*Pinus sylvestris*) som en effekt av beståndstäthet och biomekanik

Hela förteckningen på utgivna nummer hittar du på www.seksko.slu.se